Indian J. Phys. **79(6)**, 591-595 (2005)

AN INTERNATIONAL JOURNAL
IJP

Photoacoustic spectroscopy : a technique to study plant-pathogen interaction

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Received 22 November 2004, accepted 23 March 2005

Abstract : Photoacoustic spectroscopy (PAS) has been applied to study the plant-pathogen interactions and the pathological diagnosis of diseases in various plant species. The Photoacoustic (PA) spectra of fungal infected leaves and healthy leaves were recorded. The samples of the mustard (*B. juncea* var. Kranti) leaves infected with Alternaria blight, exhibited a higher PA signal strength than that of the untreated (healthy) leaves of same cultivars as well as that of the cabbage and radish. It has been observed that the PA signal strength obtained from the diseased leaves were consistently higher than that of the healthy leaves for all samples. One of the possible reasons behind this fact is the contribution of larger heat emission through the disease leaves than the healthy leaves, giving rise to higher signal strength. Our investigations also demonstrate the interaction of light radiation with the plant leaves and their pigments. The PA signal recoded from the conidia of different isolates of *Alternaria* sp. (*A. brassicae, A. brassicicola and A. raphani*) also show differences in their characteristic graphic patterns.

Keywords : Photoacoustic spectroscopy, absorption of radiation, plant pathogen interaction, *Alternaria* isolates.

PACS No. : 82.80. kq

1. Introduction

The Photoacoustic spectroscopy is an established technique for studying different physical properties of solids, semi-solids, liquids, semiconductors, biological materials *etc.* This technique is based on the optoacoustic effect (later known as photoacoustic effect) discovered by Alexander Graham Bell [1]. The primary development of this technique was led by Rosencwaig and Gersho [2]. The photoacoustic signal generated within the photoacoustic cell is produced as a result of periodic heat flow from the sample to the surrounding gas, as the sample is periodically heated by the excitation radiation. An electret/ condenser microphone attached to the sample compartment of the PA cell is used to detect the acoustic signal. The sample for studies is mounted in the sample holder in such a way that its front surfaces are exposed to the air (gas) within the cell. The boundary layer of air may be considered as a vibrating piston, which produce acoustic signal. The strength of the photoacoustic signal depends on the nature of material *viz.* density, specific heat thermal diffusivity of material. The strength of PA signal also depends on the intensity of radiation, absorption coefficient of the sample and the wavelength of the incident radiation. PA effect is basically an energy conversions process in which electromagnetic radiations are converted into heat energy and, this in turn, generates the acoustic signal.

Although many biological materials occur naturally in a soluble state, however, some are the membrane-bound and some part of the tissue structures. These materials are insoluble and function biologically within a more or less solid matrix. Optical data on these materials are generally, not in a suitable state for conventional transmission spectroscopy and when solubilized, they are often structurally altered [2]. The present study deals

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with the understanding of the plant pathogen interaction by PA technique, which can be employed for investigating the properties of such biological materials both *in-vitro* and *in-vivo*. This method is being successfully applied to understand the photo-biological responses from plants related to agriculture and many other fields. The PA technique was so far applied in photosynthesis research to detect ethylene produced by plants during senescence [3–6], to study the effect of herbicides on plants [7], to detect the fungal infections in seeds [8,9] and leaves infected by fungal and to detect the leaves' diseases [10–14].

Successful execution of disease management program depends on the understanding of pathogen population structure and mechanism by which variation arises within those populations. *A. brassicae, A. brassicicola* and *A. raphani* are the major pathogens, which attack on their hosts mustard, cabbage and radish. Therefore, we have planned to use PAS technique for early detection of disease caused by different *Alternaria* sp. *viz. A. brassicae, A. brassicicola* and *A. raphani*. The information is based on exploiting the differences of reduced photosynthesis, destruction of leaf tissue/pigments and accumulation of specific substances. In the present study, we report, the PA spectra of *Alternaria* infected leaves of different genotypes (*B. juncea* var. Kranti, *B. oleracea* var. *capitata* and *R. sativus*). The major objective to undertake the study was to exploit the potential of PAS as a diagnostic tool for detection of pathogens.

2. Materials and methods

2.1. Experiential setup :

The schematic experimental setup of PAS is shown in Figure 1. The 632.8 nm radiation of 10 mW linearly polarized He-Ne laser (510 P, Aerotech, USA) is used as a radiation source. Although He-Ne laser can operate in three different spectral regions (632.8 nm, 1150.0 nm and 3390.0 nm), its operation in red at 632.8 nm is usually expected when it is used for alignment, interferometery, data processing *etc*. The visible He-Ne laser radiation at 632.8 nm arises from $3S^2$ to $2P^4$ transition, which provides the laser action. This radiation passed through a mechanical chopper (model SR 540 OPTICAL CHOPPER), which chops the incident light radiation at the rate of 4 Hz to 4 KHz. The modulated radiation was focused into the sample compartment of an indigenously developed photoacoustic cell. The photo-acoustic cell is the most important part of the experimental arrangement. This is a thin aluminum cell body, which is acoustically isolated from the external disturbances and noises. A sample holder made of gunmetal is placed inside the cell and the light radiation fall on it through the quartz window. A sensitive condenser microphone attached to the right side of the sample holder is used to detect the PA signals. The PA responses were recorded by varying the chopping frequency from 5 Hz to 350 Hz. The signal was amplified by preamplifier (variable-gain) and then processed by a lock-in-amplifier (model # SR-530, USA). The time constant of 30 seconds was used for all measurements. The lock-in-amplifier increases the signal to noise (S/N) ratio. Lock-in-amplifier also generate reference signal for chopper by PPL (phase locked loop) circuit. The experiment is carried out at the incident radiation of 632.8 nm and the chopper frequency varied from 5 Hz to 350 Hz.

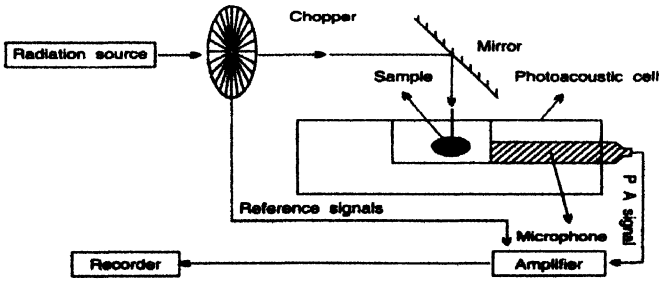

Figure 1. Schematic diagram of the photoacoustic spectrometer.

The resolution of instrument/monochromator is 5 nm. In the present investigation monochromatic light from He-Ne source has been used as the excitation energy; therefore, the resolution of the instrument does not affect the signals, strength significantly. The time constant of the frequency counter is 0.01 Hz. The PA signals depend only on chopper frequency, not in time therefore, no variation in PA signal occurs with time.

2.2. Obtaining Alternaria isolates :

Isolation from distinct leaf spots naturally produced on *B. juncea* var. Kranti (mustard), *B. oleracea* var. *capitata* (cabbage) and *R. sativus* (radish), resulted in constant recovery of three distinct *Alternaria* isolates (*A. brassicae, A. brassicicola* and *A. raphani*). Fresh isolations from the above respective category of *Alternaria* isolates were obtained separately as generation of single conidial culture on radish root mannitol agar (radish root extract 200 g, agar 20 g/l and mannitol 20 g/l) supplemented with rose

bengal (50 μg/l). All the three *Alternaria* isolates were further maintained on the above medium at 20 ± 2°C.

2.3. *Plant material :*

Mustard (*B. juncea* var. Kranti), cabbage (*B. oleracea* var. *capitata*) and radish (*R. sativus*) were taken to study host pathogen interaction. Seeds of the above genotypes were grown in plastic pots having steam-sterilized soil (loamy soil + sand + FYM in ratio of 3 : 1 : 1). For this purpose, surface sterilized seeds with 0.5% $NaOCl_2$ solution were sown in these pots and kept inside the glasshouse. 2 plants/pot, with three replications of each genotype were maintained after 20 days of sowing.

2.4. *Preparation of inoculum :*

Conidial suspension 1.5 × 10^5 ml^{-1} was prepared from 15-day-old culture of *Alternaria* isolates (*A. brassicae, A. brassicicola* and *A.raphani*) separately in sterilized distilled water. Spore suspension was passed through four layers of cheesecloth, to remove mycelial fragments and centrifuged at 3000 rpm for 5 min for removal of unwanted material. Then the conidial concentration in the inoculum was maintained for each isolate at 1.5 × 10^5 conidial ml^{-1}, with the help of haemocytometer.

2.5. *Inoculation of plants :*

Thirty-day-old plants were inoculated separately with *Alternaria* isolates (*A. brassicae, A. brassicicola* and *A. raphani*), with the inoculum as prepared above. The plants were inoculated with the atomizer. The plants were kept in the moist chamber with 90–95% RH and temperature 15 ± 2°C.The pots were well marked/tagged and kept inside the moist chamber for symptoms development. Appropriate control was also maintained by inoculating the leaves with sterilized distilled water.

2.6. *PA spectra of leaves :*

Infected leaves of mustard, cabbage and radish were collected according to different treatments in different polythene bags from the glass house and brought to the laboratory for recording the photoacoustic spectra. About 1 cm^2 area of the leaves having 10 mg weight (healthy, resistant and inoculated with different isolates of *A. brassicae, A. brassicicola* and *A. raphani*) were cut and enclosed in the photoacoustic cell for recording the photoacoustic spectra. The experimental conditions were the same for each leaf. It is pointed out that no variety has been found so far which is resistant to Alternaria blight pathogen, but some tolerant varieties are there and we are taking one such variety *B. juncea* cv. PHR-1 in our experiment. The experiment was repeated three times to get better accuracy.

PA spectra of different Alternaria isolates :

For studying PA spectra of different isolates of Alternaria, 20-day-old culture of each isolate having 5 mg weight (*A. brassicae, A. brassicicola* and *A. raphani*) was taken. The cultures were taken in 12 h light and 12 h dark conditions in the sporulating chamber so that they show profuse sporulation. The PA spectra of pathogen spores were recorded by loading the pathogen spores directly into the photoacoustic cell.

3. Results and discussion

The PA spectra of conidia of different isolates of *Alternaria viz. A. brassicae, A. brassicicola* and *A. raphani* showed variability in the photoacoustic spectrum shown in Figure 2. The observed spectral characters were

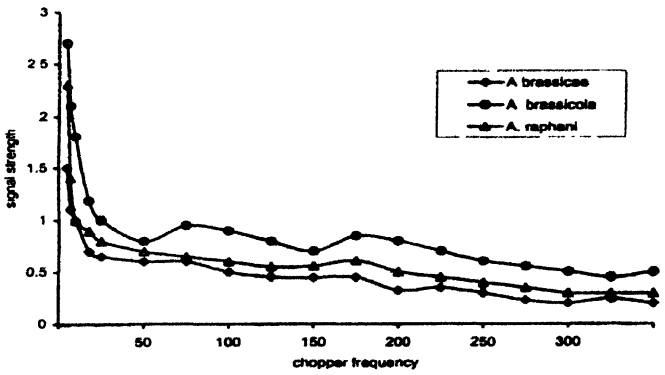

Figure 2. Photoacoustic spectra of different alternaria isolates.

markedly different for each isolate at lower frequencies between 5 Hz to 150 Hz and the strength of PA signals of different isolates was comparable, while at higher frequencies, 150 Hz to 350 Hz, the photoacoustic spectra of the isolates showed almost similar graphic pattern. The PA spectra of the three isolates of *Alternaria* show variability in lower frequencies (5 to 50 Hz) but at higher frequencies, the PA spectra did not show any remarkable change. The PA spectra of *A. brassicicola* and *A. raphani* were different than that of *A. brassicae;* their PA spectrum also showed greater differences at the lower frequencies but at the higher frequencies, they were more or less similar. Likewise, the PA spectra of the leaves of *Brassica juncea* var. Kranti, *Raphanus sativus* and *Brassica oleracea* var. *capitata* inoculated

with isolates of *A. brassicae*, *A. raphani* and *A. brassicicola* (Figure 3–5) showed a remarkable variability.

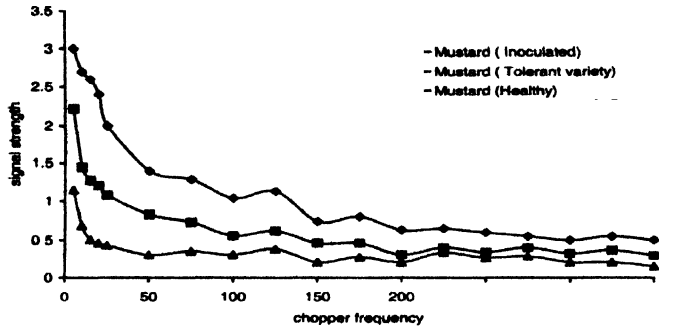

Figure 3. Photoacoustic spectra of mustard.

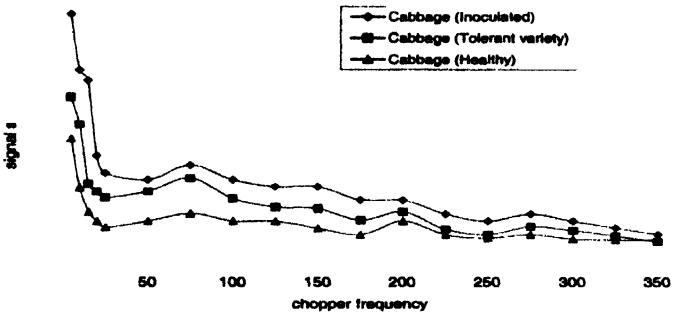

Figure 4. Photoacoustic spectra of cabbage.

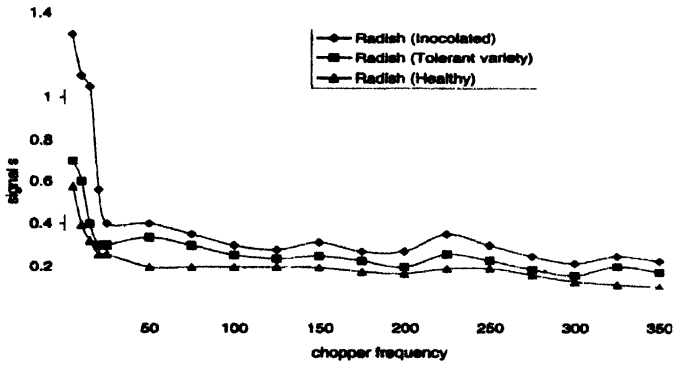

Figure 5. Photoacoustic spectra of radish.

The PA spectra of *A. brassicicola* infected leaf showed higher PA signals at the low frequency (5 to 20 Hz) but at higher frequencies, it did not show much difference. The PA spectra of *A. raphani* infected leaves also showed higher PA signals at the lower frequencies (5 to 150 Hz) but at higher frequencies, it showed less variability. The spectra of the leaves inoculated with *A. brassicae* isolate also showed differences in their spectrum. Their PA signal ranges from 0.5 to 3.0 for these isolates. The PA spectra of the healthy leaves of *Brassica juncea* var. Kranti, *Raphanus sativus* and *Brassica oleracea* var. *capitata* (inoculated with sterilized distilled water) did not show any remarkable differences in their PA spectrum. The PA signal for them ranges from 0.2 to 0.3 in every case. The PA spectra of tolerant varieties are also reported in figures. The trends of PA signals are same but it lies between the healthy and inoculated leaves.

The results of this study demonstrate that PA spectroscopy can be applied for detecting differences of the isolates. The PA spectra of *Alternaria brassicicola* and *Alternaria raphani* also showed variability in their graphic pattern. The PA graphs of leaves of different hosts (*Brassica juncea* var. Kranti, *Raphanus sativus* and *Brassica oleracea* var. *capitata*) inoculated with different isolates of *A. brassicae*, *A. brassicicola* and *A. raphani* also showed differences in their PA spectra, thus it is revealed that this technique proved useful for differential diagnosis of various isolates of same pathogen as well as different host reactions. Eventually, PA spectroscopy might be used as a rapid, sensitive and economical detection method for studying variability among pathogen. PA spectra of healthy leaves of *B. juncea* genotype Kranti showed low PA signals (0.2–0.4 volts) whereas the inoculated leaves by different isolates showed remarkable differences in their PA graphs. All the spectra of isolates showed higher PA signals at lower frequencies (5 to 50 Hz) but at higher frequencies, they did not give much difference (Figure 3).

The Alternaria blight fungus caused the destruction of photosynthetic pigments, increase in nucleic acids and changes in the macromolecules [11]. Therefore, it is possible to measure the extent of disease severity symptoms by means of photoacoustic analysis as being used to detect distribution of photosynthesis pigments in diseased leaf tissues at various depths [5]. Photoacoustic signal strength from the inoculated leaves, was consistently higher than that of healthy leaves. It is because the contribution of heat emission through the non-radiative de-excitation in the diseased leaves is large. Due to destruction of chlorophyll molecules in the diseased leaves, the absorbed photons, not trapped by the primary photosynthetic pigments, contribute to heat emission through non-radiative de-excitation, resulting in a strong photoacoustic signal. On the other hand, the healthy leaf displayed weak photoacoustic signal at all the frequencies due to utilization of photons by the photosynthetic pigments and thus, their reduced contribution to non-

radiative de-excitation. The contribution of heat emission through non-radiative de-excitation in the diseased leaves is higher due to the appearance of other biomolecules as a result of fungal infection. These new biomolecules absorb the photons and do not transfer them to the primary photosynthetic reaction center. Therefore, they emit these photons non-radiatively, which leads to enhanced photoacoustic signal as compared to those form healthy leaves. Our observations are strengthened by the work of many workers [8,12–16].

The observed spectral characteristics were markedly different for each genotype. At lower frequencies, the strength of photoacoustic signals form moderately and severely infected leaves were comparable while the healthy leaf exhibited a relatively weak photoacoustic signal [3]. During the infection process of *A. brassicae*, the host cell shows necrotic response. Once within the host, the epidermal cells are fully invaded and mycelia ramify through and between the mesophyll and palisade cells, the entire leaf is soon parasitized. Early in the post penetration phase, the invaded epidermal cells become necrotic and the parenchyma tissue ahead of the advancing hyphae often collapse. This phase shows the disappearance of chlorophylls due to disorganization of chloroplast grana [11]. Therefore, it is possible to measure the extent of disease by means of photoacoustic analysis used to detect distribution of photosynthesis pigments in diseased leaf tissues at various depths [3].

The results of present study reveal that on the basis of disease reactions of different isolates of *A. brassicae*, *A. brassicicola* and *A. raphani* genotypes, we can detect the extent of damage caused by a particular pathogen. Present study demonstrates that PAS can be a useful method for pathological diagnosis, plant pathogen interaction and screening for resistance in the breeding programs. As a procedure, it would be useful to first screen for resistance by PAS and further test the identified resistant genotypes by epidemiological components.

Acknowledgment

Authors gratefully acknowledge Dr. J C Kapil for providing with laboratory facility, technical assistance and suggestions for this work.

References

[1] A G Bell *Am. J. Sci.* **20** 305 (1880)

[2] A Rosencwaig and A Gersho *J. Appl. Phys.* **47** 64 (1976)

[3] C Buschmann *Botan. Acta* **103** 9 (1990)

[4] C Nitsch, Braslavsky and G H Schatz *Biochimica et Biophysica Acta* **934** 33 (1989)

[5] C Buschmann *Phil. Trans. Roy. Soc. Lond.* **B323** 423(1989)

[6] D Bicanic, F Harren, J Renss, E Woltering, J Snel, L A C J Voesenek, B Zuidburg, H Jalink, F Bijnin, C W P M Blom, H Sauren, M Kooijman, L Van Hove and W Tonk *Photoacoustic Processes in Gases* (ed) P Hess (Berlin : Springer-Verlag) **Vol 46** p210 (1989)

[7] Z Szigeti, E M Nagel, C Buschmann and H K Lichtenthaler *J. Plant Physiol.* **134** 104 (1989)

[8] R V Greene, S H Gordan, M A Jackson, G A Benett, J F Mc Clelland and R W Jones *J. Agric. Food Chem.* **40** 1144 (1992)

[9] S H Gordon, R W Jones, J F Mc Clelland, D T Wicklow and R V Greene *J. Agric. Food Chem.* **12** 5667 (1999)

[10] P Palaria, A K Rai and D Mathur *Instrum. Sci. Tech.* **26** 221 (1998)

[11] P R Verma and G S Saharan *Monograph on Alternaria Disease of Crucifers* (Saskatoon Research Centre Technical Bulletin, 1994-6E, Agriculture and Agri-food Canada, Saskatoon, S K Canada) (1994)

[12] P Helander, L Ingemar and D Mc Queen *J. Appl. Phys.* **6** 81 (1981)

[13] P Palaria, D Mathur and A K Rai *J. Sci. Res.* **48** (1998)

[14] C S Patni, S J Kolte and R P Awasthi *Indian Phytopath.* **57** 73 (2004)

[15] S K Singhal, K P Singh, S K Joshi and A K Rai *Curr. Sci.* **82** 172 (2002)

[16] A K Rai and D Mathur *Proc. Nat. Symp. on Recent Adv.* (Laser Molec. Spectros. DDU, Gorkhpur University, India) p44 (1998)